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A Physiologically Based Model of the HPG Axis in Fathead Minnows

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Overview

- Background
  - The endocrine system
  - Endocrine active compounds (EACs) and their sources in the environment
- Objective
- Model Development
- Results
- Conclusions

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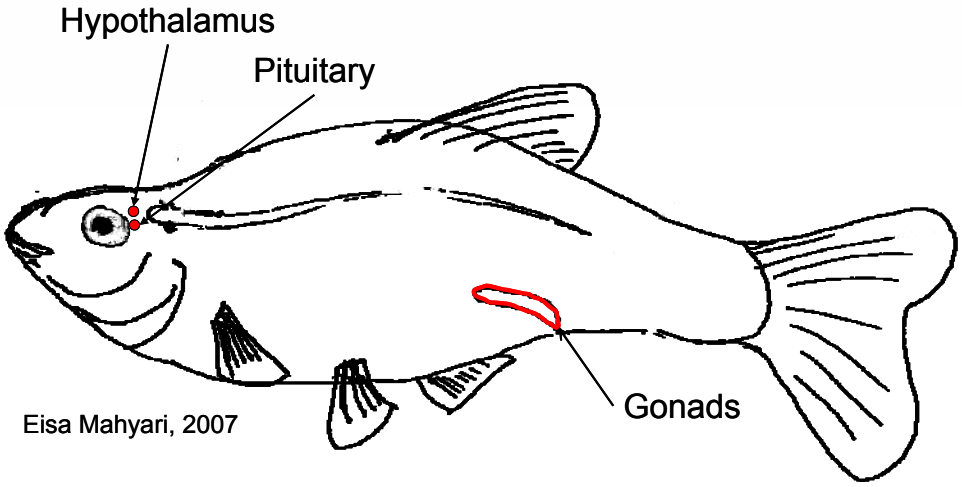
The Endocrine System

- A network of glands distributed throughout the body
  - Hypothalamus
  - Pituitary
  - Pineal gland
  - Thyroid
  - Parathyroid
  - Adrenal
  - Pancreas
  - Gonad: Ovary or Testis
- These glands produce hormones that are distributed to distant target sites via the blood.



GreenFacts (2006). http://www.greenfacts.org/endocrine-disruptors/endocrine-disruptors.htm#1

What are EACs?

- Chemicals, both natural and man-made, that can interfere with endocrine glands and their hormones or where the hormones act, i.e., the target tissues
- Environmental sources
  - Sewage treatment plant effluents
  - Pulp and paper mill effluents
  - Marine paints – tributyltin
  - Fungicides – vinclozolin and linuron
  - Herbicides – chlorotriazines such as atrazine
  - Plastics manufacturing - phthalates
  - Livestock industry pharmaceuticals

Daston et al. (2003). Toxicological Sciences 74(2): 245-252.

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Effects in Wildlife

- Adverse effects observed in certain fish and wildlife
  - Estrogen exposure biomarker, vitellogenin, found in fish living near outflows from sewage treatment plants or pulp and paper mills
  - Imposex in molluscs exposed to tributyltin
  - Reproductive and immunological effects in marine mammals (PCBs and DDE)
  - Egg shell thinning and embyronic abnormalities in birds (DDT and PCBs)

Damstra et al. (2002) Global Assessment of the State-of-the-Science of Endocrine Disruptors. Daston et al. (2003) Toxicological Sciences 74(2): 245-252.

Risk Assessment Needs

- Methods to predict effects of EACs on populations
- Tools to extrapolate effects from high to low dose
  - Target tissue dosimetry
  - Developing quantitative relationships between gene expression and higher order responses
- Identification of biomarkers as indicators of exposure and predictors of adverse effects
- Tools to extrapolate across species
  - Similarities across vertebrates for certain processes, e.g., steroidogenesis
  - Differences expected in ligand-receptor binding

Daston et al. (2003). Toxicological Sciences 74(2): 245-252.

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Objective

- Develop a physiologically based computational model to simulate reproductive endpoints in male fathead minnows (FHM, *Pimephales promelas*) exposed to 17α-ethynylestradiol (EE_2)

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Reproductive Endpoints of Interest

- Steroid hormones
  - Testosterone (T)
  - 11-ketotestosterone (KT)
  - 17β-estradiol (E_2)
- Vitellogenin (Vtg)
  - Pre-cursor to a major egg yolk protein
  - Normally found at low levels in male FHM
  - Biomarker of exposure to estrogenic EACs because Vtg is induced after exposure

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Why Model Fathead Minnows?

- Distributed throughout North America
  - Fresh water habitat
- Sexually dimorphic
- Fractional spawners
  - Spawn every 3-5 days
- Mature in 4-6 months
- EPA model for toxicity

Photo courtesy U.S. EPA

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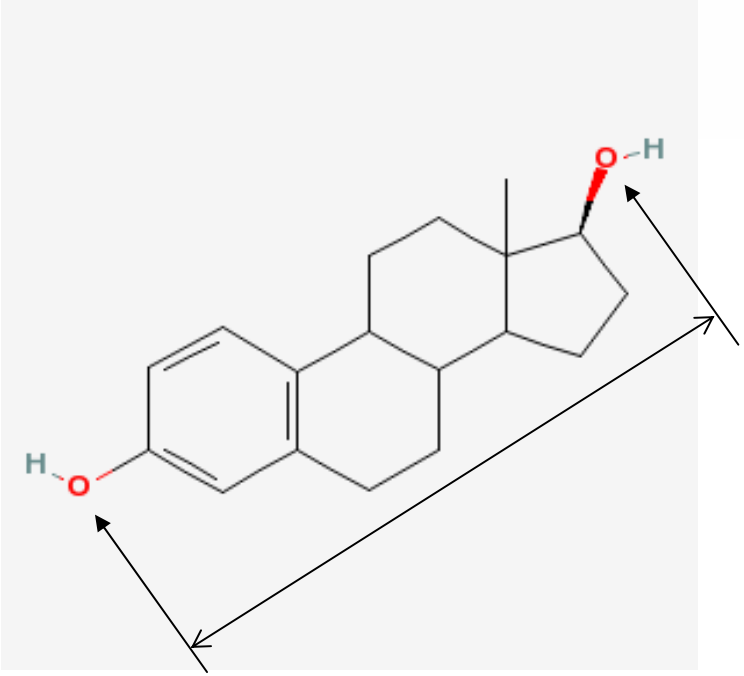
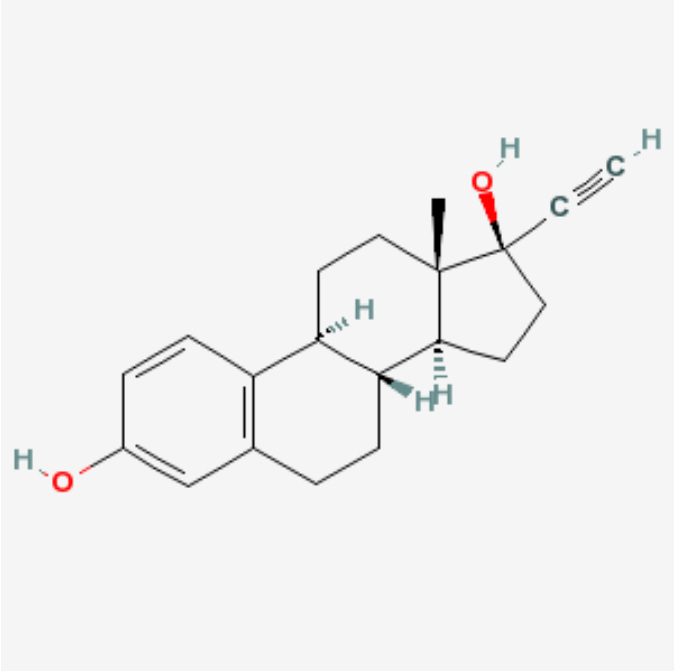
Why EE_2?



E_2

An estrogen synthesized naturally



EE_2

Synthetic estrogen used in oral contraceptives

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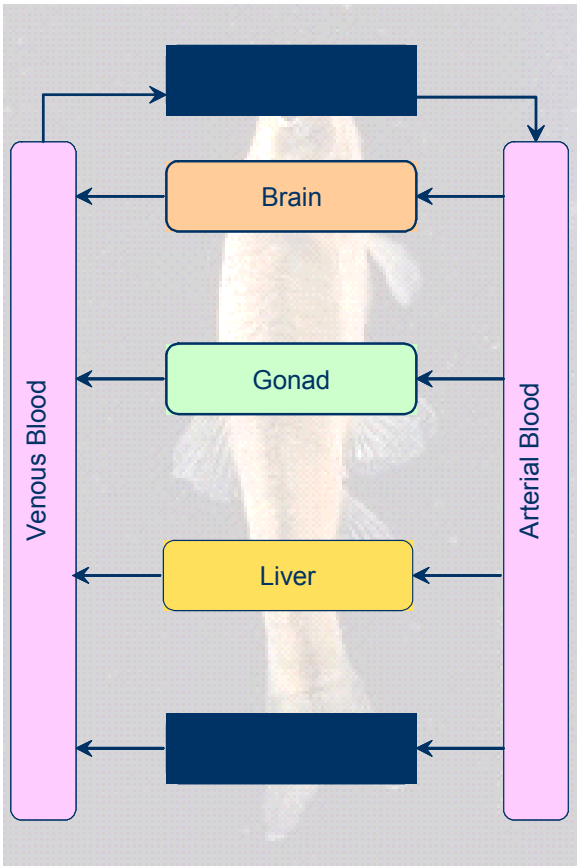
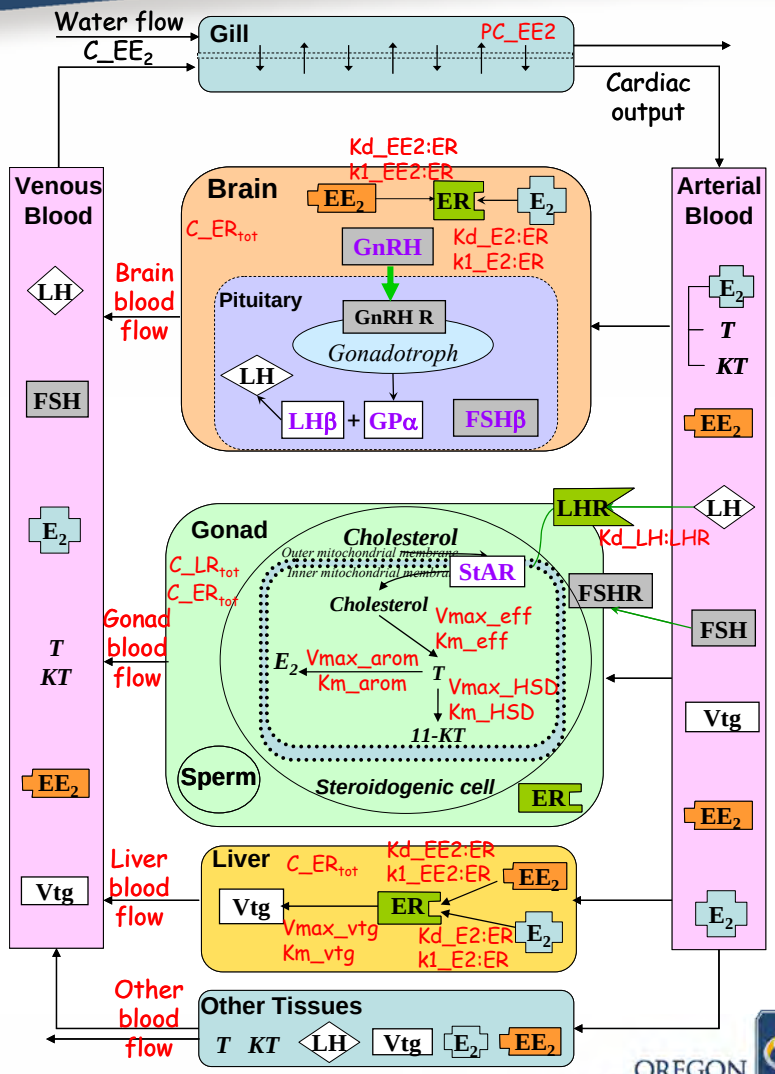
Conceptual Model of the HPG Axis





Adapted from Villeneuve et al. (2007). Environmental Science & Technology 41(1): 321-330.

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Physiologically Based Model Formulation

- Mass balance

  F = volumetric flow

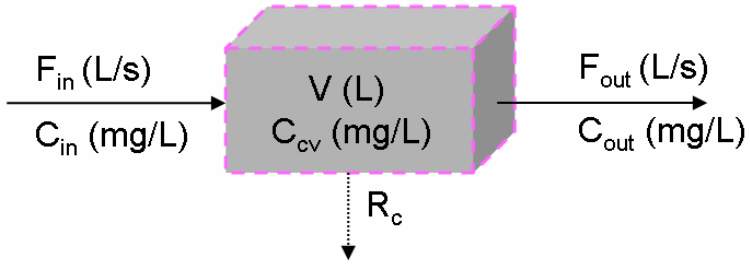
  C = concentration

  V = tissue compartment volume

  R = rate of consumption (e.g., metabolism or binding), or a rate of production preceded by a “+” sign

  t = time

$$\text{Accumulation} = \Sigma \text{ Flow in} - \Sigma \text{ Flow out} + \Sigma \text{ Production} - \Sigma \text{ Consumption}$$



$$\frac{d(C_{cv}V)}{dt} = F_{in}C_{in} - F_{out}C_{out} - R_c$$

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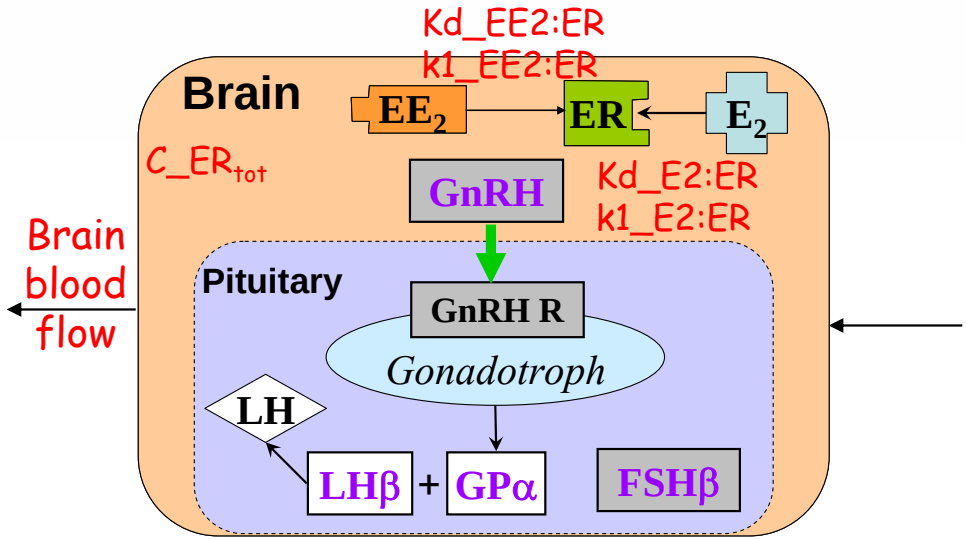
Brain

$$\frac{d(V_{brn}\,C_{EE2,brn})}{dt} = F_{brn}\,(CArt_{EE2} - CVen_{EE2,brn}) - V_{brn}\,(k1_{EE2,brn}\,C_{EE2,brn}\,C_{ERf,brn} - k2_{EE2:ER,brn}\,C_{EE2:ER,brn})$$

$$\frac{d(V_{brn}\,C_{E2,brn})}{dt} = F_{brn}\,(CArt_{E2} - CVen_{E2,brn}) - V_{brn}\,(k1_{E2,brn}\,C_{E2,brn}\,C_{ERf,brn} - k2_{E2:ER,brn}\,C_{E2:ER,brn})$$

$$\frac{d(V_{brn}\,C_{LH,brn})}{dt} = F_{brn}\,(CArt_{LH} - CVen_{LH,brn}) + \rho_{LH,brn}\,C_{E2,brn}\,V_{brn} + \dot{m}_{LH,brn}$$



Similar equations are formulated for T, KT, and Vtg

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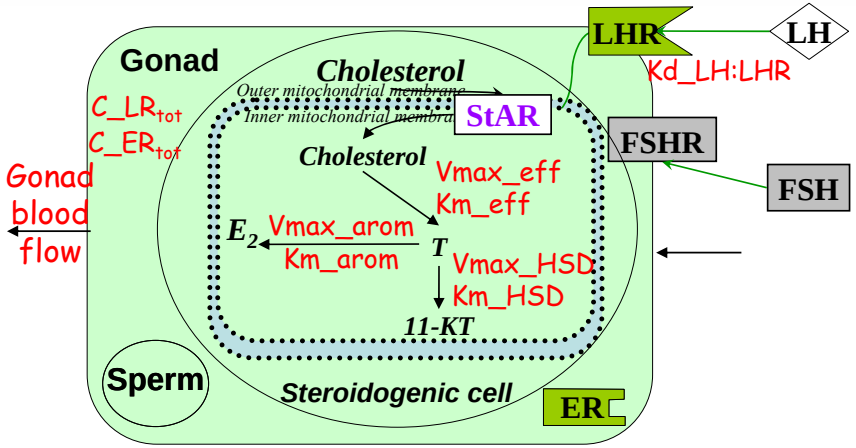
Gonad

$$\frac{d(V_{\text{gon}}\, C_{\text{EE2,gon}}\,)}{dt} = F_{\text{gon}}\,(\text{CArt}_{\text{EE2}} - \text{CVen}_{\text{EE2,gon}})$$
$$- V_{\text{gon}}\,(\text{k1}_{\text{EE2,gon}}\, C_{\text{EE2,gon}}\, C_{\text{ERf,gon}}$$
$$- \text{k2}_{\text{EE2:ER,gon}}\, C_{\text{EE2:ER,gon}}\,)$$

$$\frac{d(V_{\text{gon}}\, C_{\text{E2,gon}}\,)}{dt} = F_{\text{gon}}\,(\text{CArt}_{\text{E2}} - \text{CVen}_{\text{E2,gon}})$$
$$- V_{\text{gon}}\,(\text{k1}_{\text{E2,gon}}\, C_{\text{E2,gon}}\, C_{\text{ERf,gon}}$$
$$- \text{k2}_{\text{E2:ER,gon}}\, C_{\text{EE2:ER,gon}}\,)$$
$$+ \text{Vmax}_{\text{arom}} C_{\text{T,gon}} / (\text{Km}_{\text{arom}} + C_{\text{T,gon}})$$

$$\frac{d(V_{\text{gon}}\, C_{\text{LH,gon}}\,)}{dt} = F_{\text{gon}}\,(\text{CArt}_{\text{LH}} - \text{CVen}_{\text{LH,gon}})$$
$$- V_{\text{gon}}\,(\text{k1}_{\text{LH,gon}}\, C_{\text{LH,gon}}\, C_{\text{LR,gon}}$$
$$- \text{k2}_{\text{LH:LR,gon}}\, C_{\text{LH:LR,gon}}\,)$$



Similar equations are formulated for T, KT, and Vtg

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Model Calibration Data

- Measurements for 154 unexposed male FHM (Dr. Ankley's Lab, U.S. EPA)
  - Body weight
  - Gonad weight
  - Liver weight
  - Reproductive endpoints include plasma concentrations of
    - E_2
    - T
    - KT
    - Vtg
- EE_2 48 hour exposure experiment
  - 50 ng/L in water
  - Measurements of
    - EE_2 concentration in pooled carcass and liver
    - Plasma T and Vtg concentrations

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Model Input Parameter Estimation

- Rationale for parameter estimation by Markov Chain Monte Carlo (MCMC) simulation
  - Incorporates prior knowledge about the input parameters
  - Accounts for covariance between model input parameters
  - Results in a distribution of model parameters that can be used to quantify uncertainty in model predictions
- Computational requirements
  - Software: MCSim is available freely through GNU
  - Operating system: LINUX or UNIX

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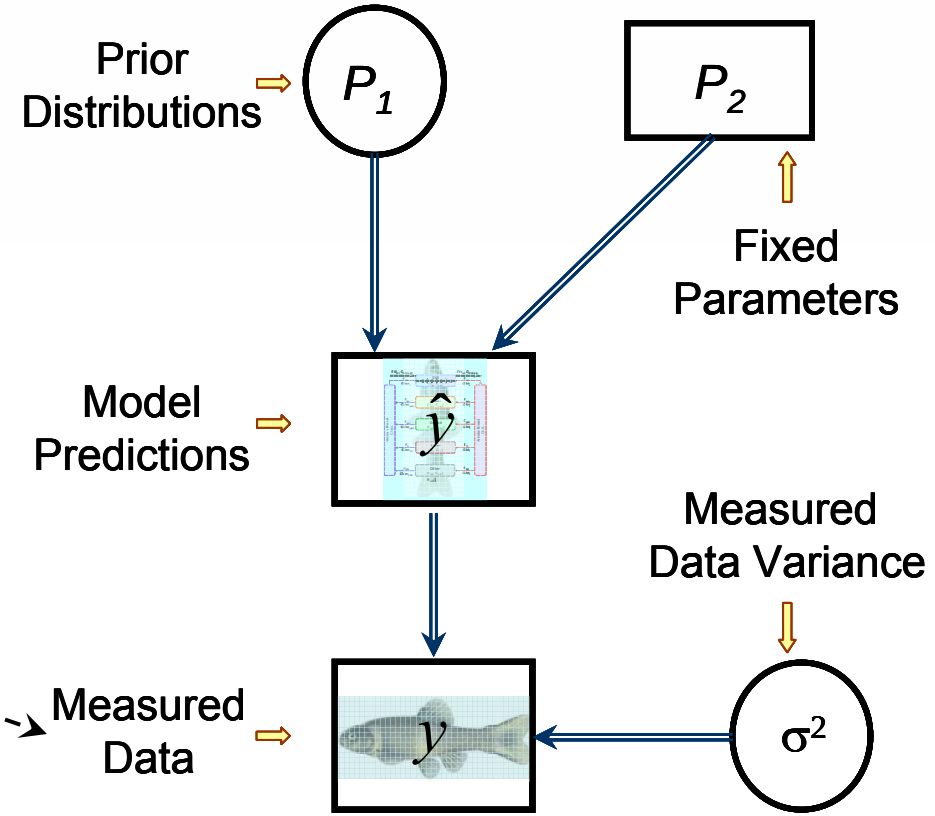
Markov Chain Monte Carlo

- 15 parameters estimated
  - Prior distributions informed by the literature
- Data from EE_2 exposed and unexposed FHM
  - Measured model outputs, y_j
    - E_2
    - T
    - Vitellogenin
    - EE_2



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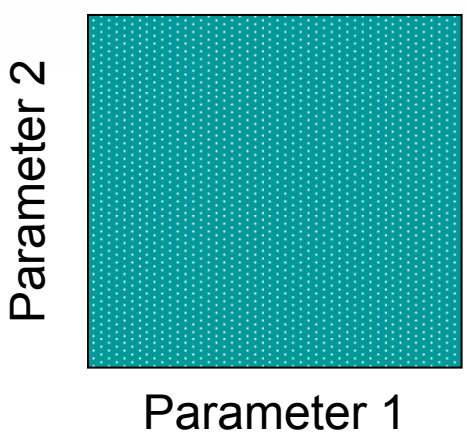
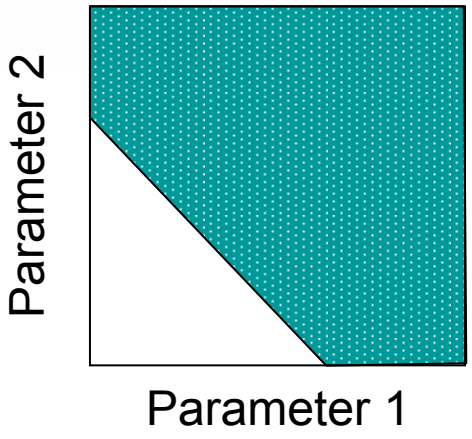
From Prior to Posterior Distributions

Prior distribution + Data => Posterior Distribution





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Model Calibration

- Data
  - Half of the unexposed FHM data were used
  - All of the EE_2 experimental measurements were used
- MCMC simulation
  - 15 model parameters were calibrated
  - Four Markov chains were simulated containing 2500 iterations each
    - 1000 iterations required ~24 hours
    - Last 500 iterations from each chain were saved
    - Convergence evaluated using the potential scale reduction metric (Gelman et al., 1995)

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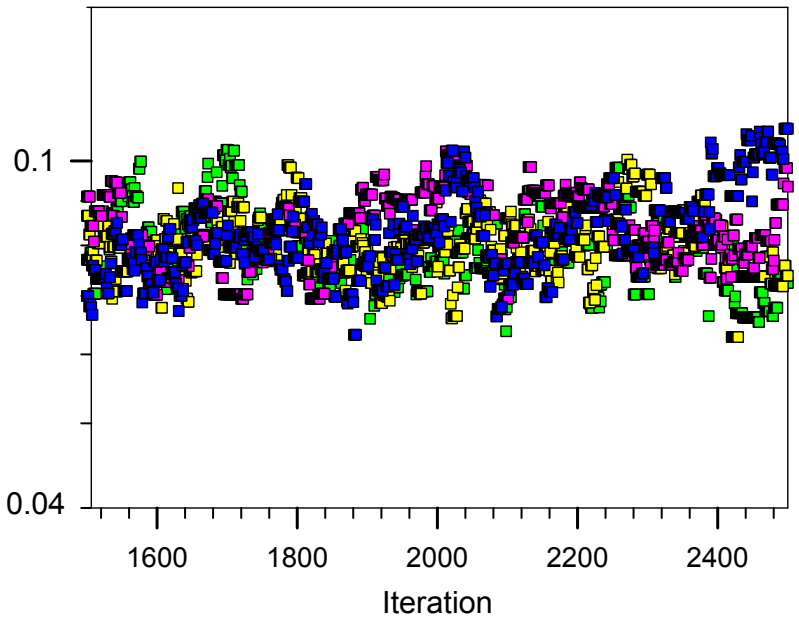
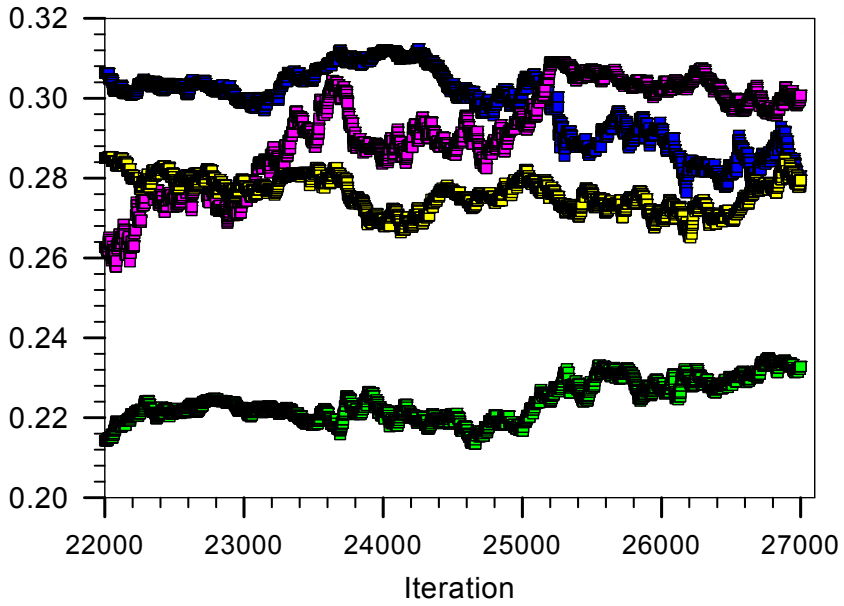
Visualizing Convergence

Chains converge
Potential scale reduction = 1.0



Chains do not converge
Potential scale reduction = 4.2



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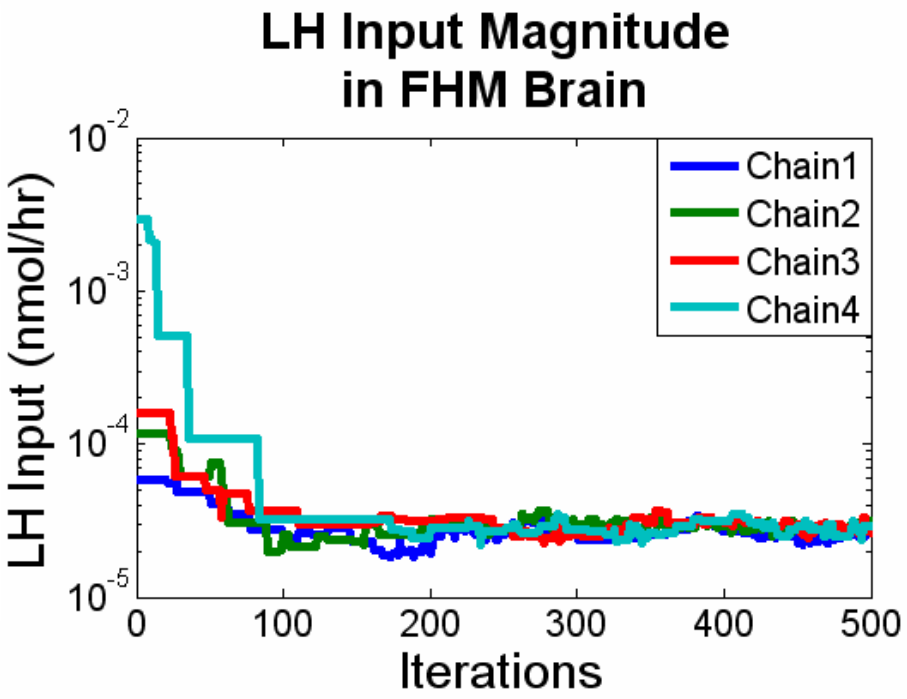
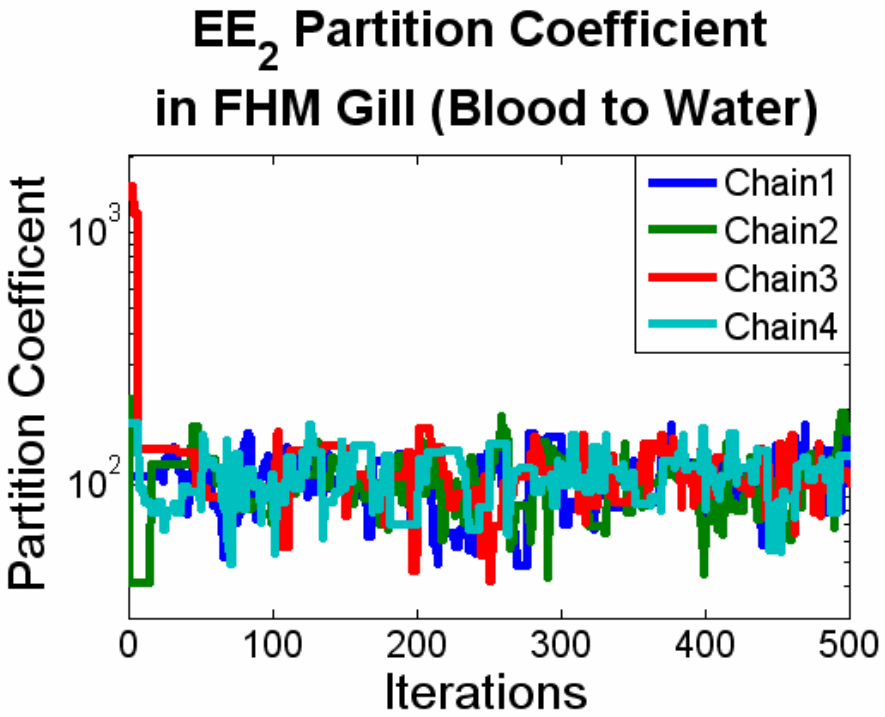
Results: Parameter Estimation





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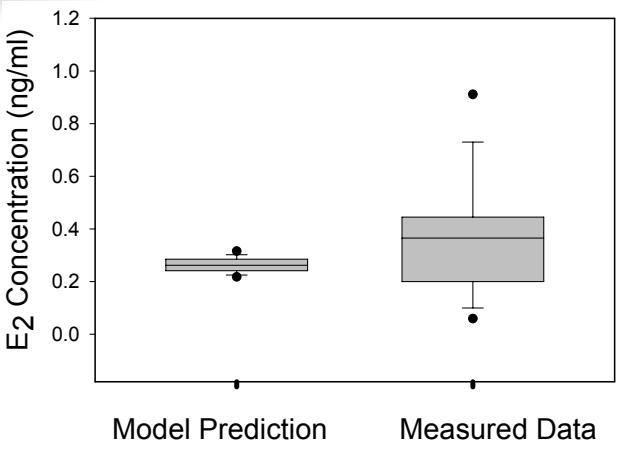
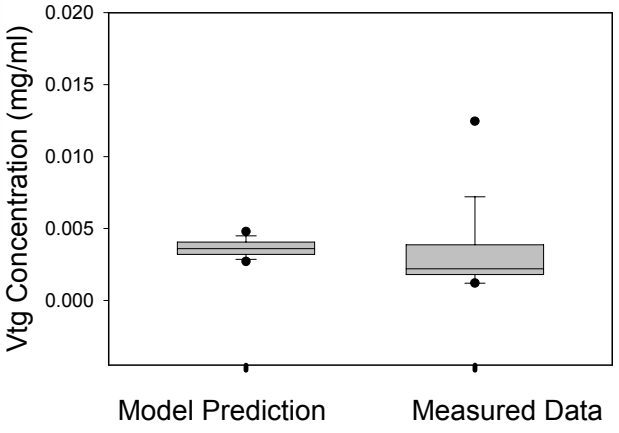
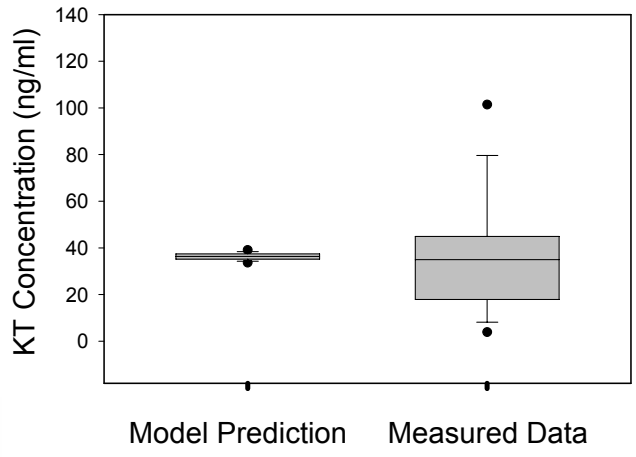
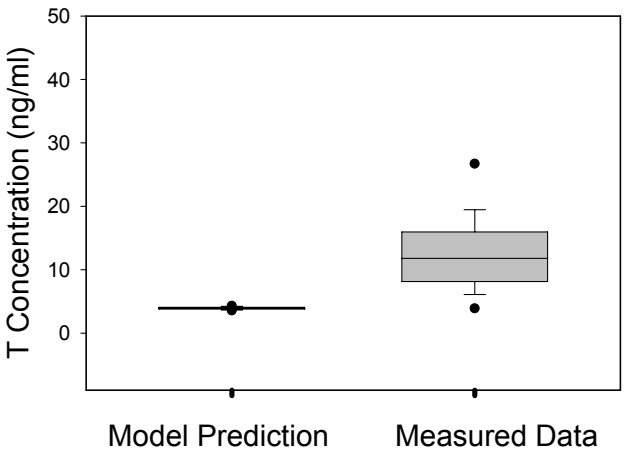
Results: Model Verification









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Summary

- There is good agreement between median model predictions of plasma E_2, KT, and Vtg concentrations
- The model underpredicts median plasma T concentrations
- The variance in model predictions for all four reproductive endpoints is lower than what is observed

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